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TITLE: Targeting Mitochondrial Inhibitors for Metastatic Castrate-Resistant Prostate Cancer

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14. ABSTRACT
The overarching challenge and focus area for this Partnering PI-Idea Development Award proposal is to rapidly develop novel therapeutic agents and validate these in pre-clinical studies needed to initiate clinical development of these agents for metastatic castrate resistant prostate cancer (mCRPC). The hypothesis of the present proposal is that an innovative and effective therapeutic approach is possible by covalently coupling niclosamide and 7 hydroxy-β-Lapachone (7OH β-Lap) analog lipophilic mitochondria toxins (MT) to human serum albumin (HSA) via a PSA specific peptide linker sequence to systemically deliver these novel agents via the blood so that these cell penetrant MTs are restrictively released only via enzymatically active PSA within extracellular fluid (ECF) at sites of mCRPC. The advantage of ECF hydrolysis is that only a fraction of cancer cells need to secrete PSA since its enzymatic activity amplifies the level of liberated cell penetrant MTs within the ECF shared by all cells within the metastatic site overcoming the problem of tumor cell heterogeneity by inducing a substantial "bystander effect

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None provided

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Introduction

The overarching challenge and focus area for this Partnering PI-Idea Development Award proposal is to rapidly develop novel therapeutic agents and validate these in pre-clinical studies needed to initiate clinical development of these agents for metastatic castrate resistant prostate cancer (mCRPC). The hypothesis of the present proposal is that an innovative and effective therapeutic approach is possible by covalently coupling niclosamide and 7 hydroxy-β-Lapachone (7OH β-Lap) analog lipophilic mitochondria toxins (MT) to human serum albumin (HSA) via a PSA specific peptide linker sequence to systemically deliver these novel agents via the blood so that these cell penetrant MTs are restrictively released only via enzymatically active PSA within extracellular fluid (ECF) at sites of mCRPC. The advantage of ECF hydrolysis is that only a fraction of cancer cells need to secrete PSA since its enzymatic activity amplifies the level of liberated cell penetrant MTs within the ECF shared by all cells within the metastatic site overcoming the problem of tumor cell heterogeneity by inducing a substantial "bystander effect".

Keywords

Metastatic castration resistant prostate cancer, mitochondria toxins, human serum albumin, PSA-activated prodrugs

Accomplishments

- **What were the major goals of the project?**

Specific Aim 1. Synthesis of HSA-coupled PSA cleavable niclosamide payload-1.
Specific Aim 2. Synthesis of HSA-coupled PSA cleavable 7OH-β-Lapachone Payload-2
Specific Aim 3. Evaluate each of HSA-couple PSA cleavable MT payloads for: 1) efficiency of PSA cleavage; 2) *in vitro* therapeutic efficacy as monotherapy vs. combinational therapy against a series of human mCRPC cell lines, 3) *in vivo* therapeutic efficacy vs. host toxicity as monotherapy vs. combinational therapy against a series of human mCRPC xenografts growing both subcutaneously and within the tibia; and 4) plasma vs. tissue biodistribution.

- **What was accomplished under these goals?**

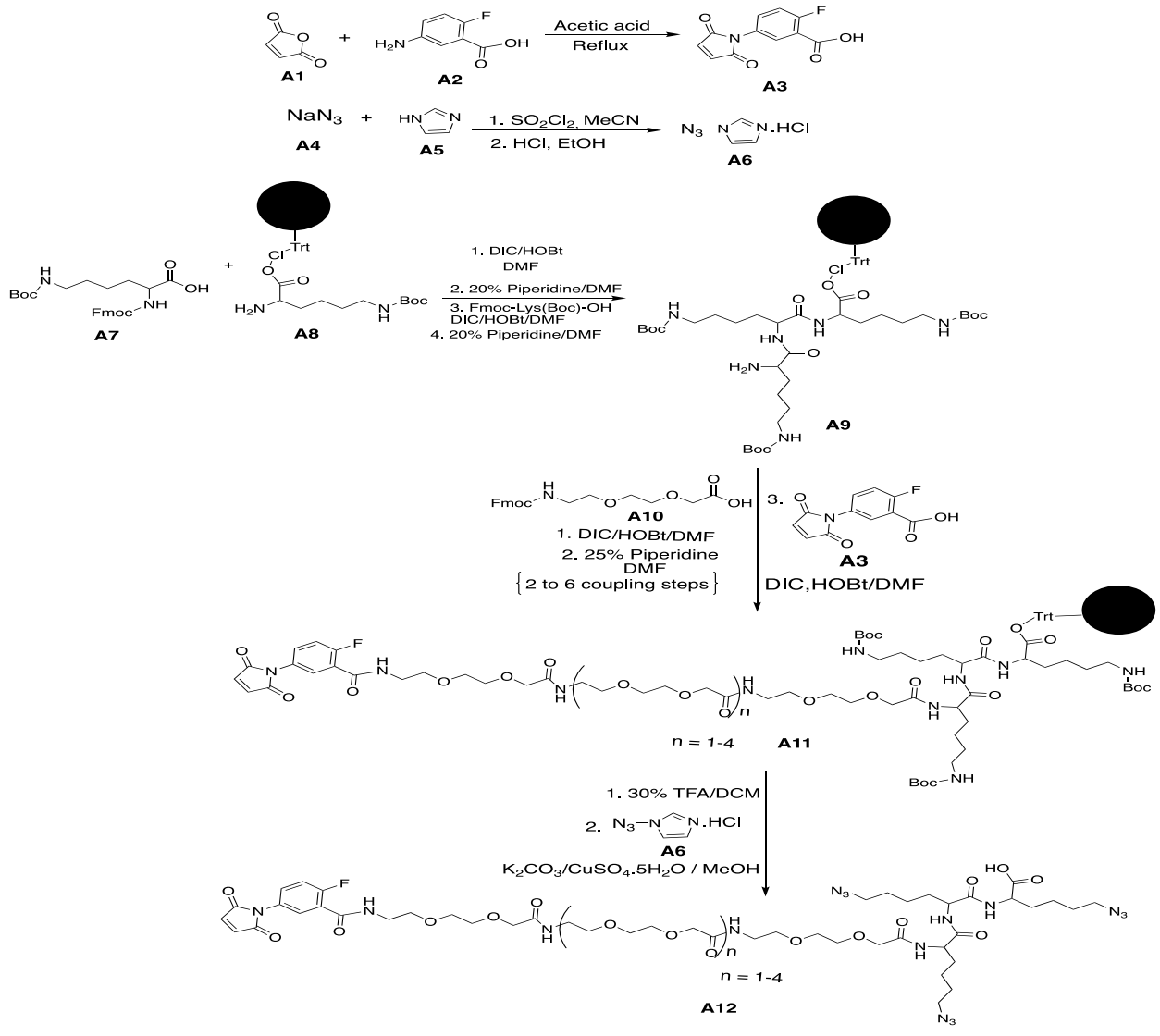
During the first year of support we have made significant progress with regard to **specific aim 1** in synthesizing a PSA activated prodrug of niclosamide coupled to human serum albumin (HSA) to obtain the final drug-HSA conjugate (i.e. **A15 in figure 2)**. This requires initial synthesis of HSSKLQLP-(SCL)-Niclosamide (i.e. **payload 1** aka **A14 in Figure 2**) and human serum albumin (HSA) covalently bound via its cysteine residue in position 34 to a PEG based linker ending in a trivalent azide side chain (i.e. **A13 in Figure 2**) followed by coupling of 3 molecules of **A14** per molecule of **A13** to produce **HSA-coupled payload 1(i.e. A15 in Figure2)**.
We have synthesized LP-(SCL)-Niclosamide in good yield using ethylene diamine derivatives as the self-cleaving linkers (SCL) and LP as dipeptide substrate for DPPIV and have scaled up the synthesis of LP-(SCL)-Niclosamide to obtain gram quantities that have been coupled to the PSA substrate HSSKLQLP (i.e. HSSKLQLP-(SCL)-Niclosamide aka **A14 in figure 2**).

To synthesize **A13**, the stable maleimide component **A3 in Figure 1** was prepared. Also, the azide transfer reagent **A6** was prepared. Both **A3** and **A6** were synthesized in good yield, and very reproducible. They were characterized with proton and carbon-13 NMR. Using a chlorotrityl resin solid phase support system, we synthesized the tripeptide molecule containing three lysine molecules. While the α-amino acid was protected with Fmoc, the ε-amino terminal of the lysine

was protected with Boc. The Fmoc of the N-terminal of the tripeptide was also removed with 20% piperidine in DMF to give **A9**. The tripeptide **A9** was characterized with MALDI-MS. To the N-terminal of **A9** was coupled {2-[2(Fmoc-amino)ethoxy]ethoxy}acetic acid using DIC and HOBt as the coupling reagent. The purpose of this is to incorporate a PEG like molecule as a linker/spacer to provide room for the coupling of the **A9** to HSA without any steric hindrance. We have used 2 to 6 units of these PEG-2 units between **A9** and the maleimide molecule **A3** so that we can investigate the effect of the length on both the coupling reaction and the kinetics of the cleavage/activation of the final bioconjugate prodrug.

To accomplish this, we began by incorporating two units of the PEG-2 derivative,{2-[2(Fmoc-amino)ethoxy]ethoxy}acetic acid, followed by Fmoc removal, and then the coupling of maleimide derivative **A3** to obtain **A11** where n = 1. This was followed by the deprotection of Boc with 30% TFA in dichloromethane, and the purified molecule was treated with the azide transfer reagent **A6** to obtain **A12** with n = 1. **A12** was characterized with MALDI-MS and HPLC. The reaction system for the **A12** synthesis is reproducible and has been scaled up to obtain gram quantity as stock for conjugation to HSA.
Currently we are conjugating **A12** to the Cysteine-34 of HSA to produce **A13** which is being coupled to **A14** to produce **A15** aka **HSA-coupled PSA activated niclosamide (i.e. Payload-1).**

Figure 1



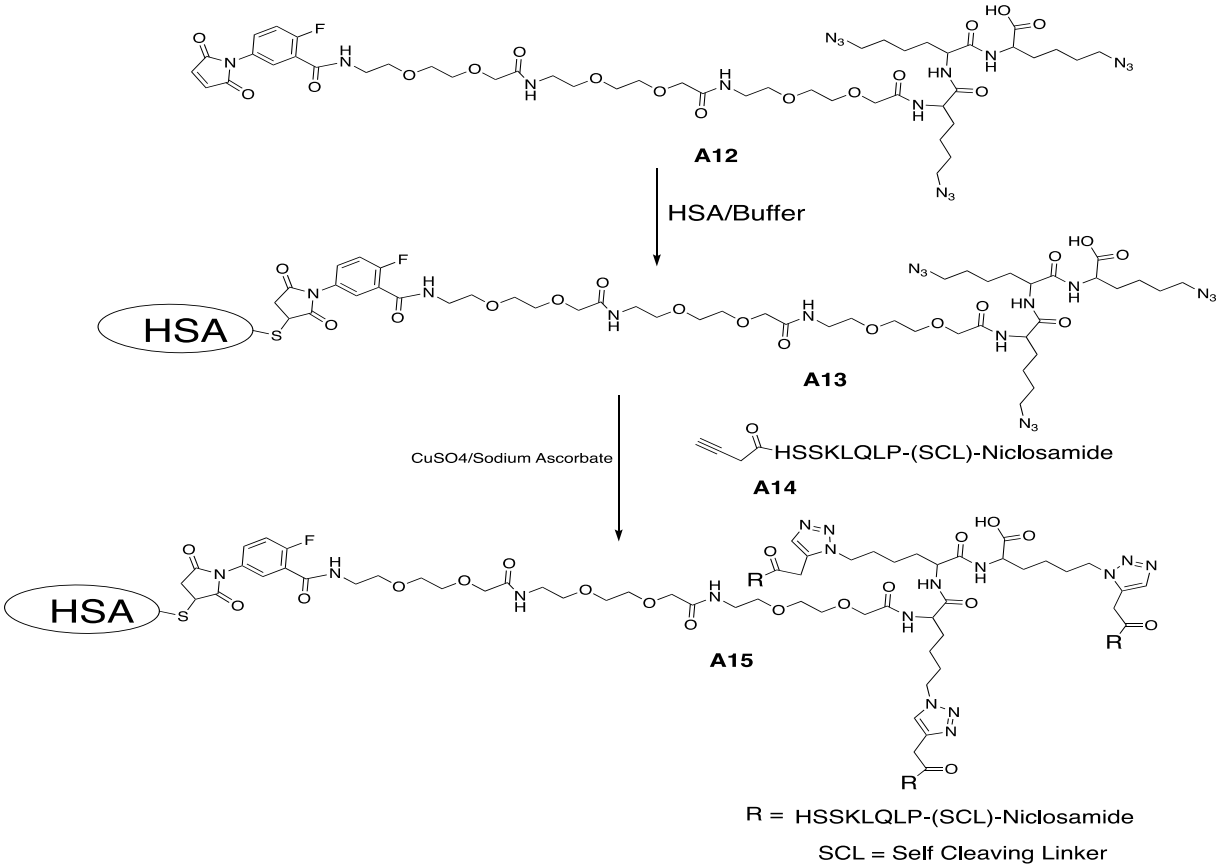


Figure 2

- **What opportunities for training and professional development has the project provided?**

Nothing to Report

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- **How were the results disseminated to communities of interest?**

Nothing to Report

- **What do you plan to do during the next reporting period to accomplish the goals?**

During the next year 7OH-β-Lapachone (7OH-β-Lap) will be synthesized as described previously, authenticity documented by ^{1}H and ^{13}C NMR and mass spectrometry, and coupled using ethylene diamine derivatives as the self-cleaving linkers (SCL) and LP as dipeptide substrate for DPPIV as described above to obtain gram quantities of LP-(SCL)- 7OH-β-Lap for coupling to the PSA substrate HSSKLQLP (i.e. HSSKLQLP-(SCL)-7OH-β-Lap.
Three molecules of resulting HSSKLQLP-(SCL)-7OH-β-Lap will be coupled to one molecule of A13 as outlined above to produce **HSA-coupled PSA activated 7OH-β-Lapachone (Payload-2).**

Each of the HSA-couple PSA activated MT payloads will be evaluated for: 1) efficiency of PSA hydrolysis; 2) *in vitro* therapeutic efficacy as monotherapy vs. combinational therapy against a series of human mCRPC cell lines, 3) *in vivo* therapeutic efficacy vs. host toxicity as monotherapy vs. combinational therapy against a series of human mCRPC xenografts; and 4) plasma vs. tissue biodistribution.

Impact

- **What was the impact on the development of the principal discipline(s) of the project?**

Nothing to Report

- **What was the impact on other disciplines?**

Nothing to Report

- **What was the impact on technology transfer?**

Nothing to Report

- **What was the impact on society beyond science and technology?**

Nothing to Report

Changes/Problems

Nothing to Report

Products

Nothing to Report

Participants & Other Collaborating Organizations

Name:	*John Isaacs*
Project Role:	*Principal Investigator*
Nearest person month worked:	*3*
Contribution to Project:	*Dr. Isaacs has coordinated/ supervised all aspects of this project on a daily basis.*
Funding Support:	*Not applicable*

Name:	*Samuel Denmeade*
Project Role:	*Partnering Principal Investigator*
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Denmeade is a collaborator in coordinating supervising all aspects of this project, including the collection and analyzes of the data. He mentors Dr. Akinboye.*
Funding Support:	*Not applicable*

Name:	*Susan Dalrymple*
Project Role:	*Sr. Research Specialist*
Nearest person month worked:	*6*

Contribution to Project:	*Ms. Dalrymple performs all routine quality assurance testing on cell lines used in this study. In addition, she has coordinated all aspects of the animal studies.*
Funding Support:	*Not applicable*

Name:	*Emmanuel Akinboye*
Project Role:	*Postdoctoral Fellow*
Nearest person month worked:	*12*
Contribution to Project:	*Dr. Akinboye synthesizes all of the compounds proposed in this application. He uses 1H and 13C NMR and mass spectrometry for the quality assurance for each compound. He also performs the mass spect analysis in the drug biodistribution studies.*
Funding Support:	*Not applicable*

Special Reporting Requirements

Partnering PI will submit his separate progress report.

Appendices

None